Enantioselective Epoxidation of ω-Bromo-2-methyl-1-alkenes Catalyzed by Chloroperoxidase. Effect of Chain Length on Selectivity and Efficiency

Frederick J. Lakner, Kevin P. Cain, and Lowell P. Hager*

Department of Biochemistry
University of Illinois
Urbana, Illinois 61801

Received August 26, 1996

Since the discovery of chloroperoxidase (CPO, EC 1.11.1.10, from *Caldariomyces fumago*) as an epoxidation catalyst,[1] the selectivity and efficiency of this reaction has been the subject of considerable interest. Our previous work regarding the asymmetric epoxidation of alkenes using CPO has entailed an initial screening of olefins,[2] evaluation of 2-methyl-1-alkenes as substrates,[3] and the utilization of CPO as a key step in the synthesis of (*R*)-(−)-mevalonolactone.[4] Zaks and Dodds[5] have expanded the scope of acceptable olefin substrates, while also demonstrating the ability of CPO to catalyze allylic and benzylic hydroxylation.[6] Indeed, CPO is a quite versatile catalyst, having been shown to mediate halogenations,[7] sulfoxidations,[8] dealkylations of alkylamines,[9] dimerization of phenols,[1a,10] and the oxidations of alcohols to aldehydes,[6,7c,11] aldehydes to acids,[5] and amines to nitroso compounds.[12]

The crystal structure[13] of CPO has been determined, and its oxidation mechanism has been investigated,[5,14] though not unequivocally determined. The enzyme is readily available and shows considerable promise as a catalyst for preparatory purposes. Most of the work so far with CPO-mediated epoxidation has been exploratory and not very systematic. A notable exception is the epoxidation of substituted styrenes.[15] In order to further probe scope and limitations and ultimately active site characteristics, this paper investigates the effect of chain length of 2-methyl-1-alkenes on enantioselectivity and conversion when subjected to CPO mediated epoxidation using *tert*-butyl hydroperoxide as terminal oxidant.

While many CPO-mediated reactions involve H_2O_2 as the terminal oxidant, this study utilizes *tert*-butyl hydroperoxide instead. The reason is primarily convenience. While CPO is able to generate O_2 from H_2O_2 in a catalase-type side reaction,[16] causing foaming and potentially sweeping away more volatile substrates, the *tert*-butyl hydroperoxide reaction can be performed in a sealed vessel without appreciable pressure buildup. Furthermore, CPO is quite sensitive to H_2O_2,[17] losing activity rapidly in the presence of excess reagent. In contrast, epoxidations run smoothly with *tert*-butyl hydroperoxide even when excess oxidant is present at the outset. Especially for larger scale reactions, the use of *tert*-butyl hydroperoxide appears more efficacious.

2-Methyl-1-alkenes were epoxidized under identical conditions: Alkene (0.30 mmol) was stirred vigorously with *t*-BuOOH (0.60 mmol) in 3.0 mL of 10 mM Na citrate buffer adjusted to pH 5.5. CPO (2.5 mg, 0.06 μmol) was added all at once. The reaction vial was capped, and the reaction was stirred for 1.0 h at ambient temperature, after which $Na_2S_2O_3$ was added and the mixture was extracted 3 times with ether. Combined organic extracts were dried with $MgSO_4$, and GC standard (decane, dodecane, or tetradecane as appropriate) was added prior to injection.

In all cases the predominant enantiomer produced was of the *R*-configuration, except 3-bromo-2-methylpropene oxide which was predominantly *S* only because of the priority switch. The enantiomer of this latter compound was synthesized from commercially available (*S*)-methylglycidol to confirm stereochemistry. (*R*)-2-Methylheptene oxide has previously been prepared[4] and correlated directly to the 7-bromo compound by hydrogenolysis of the bromide. All other epoxides were assigned stereochemistry based on their consistent elution order during GLC analysis.

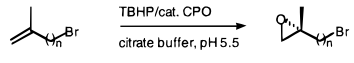
Substrate selectivity was approached by observing the effect of chain length of ω-bromo-2-methylalkenes on substrate conversion. Entries 1 and 2 in Table 1 (the two smallest alkenes) were entirely converted to products, since no starting material could be observed in reaction mixture extracts. Entries 3−5 illustrate a rapid decline in conversion with each additional carbon. For these latter three substrates that failed to convert completely, some attempts were made to increase conversions (data not shown). Doubling the initial quantity of CPO did not improve conversion. Likewise, extending reaction times to 3 h had no significant effect. On the other hand, 7-bromo-2-methylheptene was treated with an additional 2.5 mg aliquot of CPO after 0.5 h, and the reaction was quenched after another 0.5 h to reveal that conversion was improved modestly from 20% to 30%. Another increment of improvement to 36% conversion was obtained when 5.0 mg of CPO was added in four 1.25 mg aliquots at 15 min intervals. Clearly the enzyme is being inactivated rather rapidly.

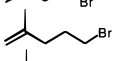
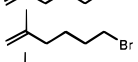
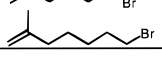
CPO activity is lost in the presence of oxidizing agents,[17] presumably while the enzyme is in its compound I form, that is, possessing a heme radical cation coordinating Fe^{IV}═O functionality. Its best protection against self-destruction appears to be the oxidation of substrate molecules, thus relieving compound I of the two electrons that may be regarded as

(1) McCarthy, M. B.; White, R. E. *J. Biol. Chem.* **1983**, *258*, 9153−8.
(2) Allain, E. J.; Hager, L. P.; Deng, L.; Jacobsen, E. N. *J. Am. Chem. Soc.* **1993**, *115*, 4415−6.
(3) Dexter, A. F.; Lakner, F. J.; Campbell, R. A.; Hager, L. P. *J. Am. Chem. Soc.* **1995**, *117*, 6412−3.
(4) Lakner, F. J.; Hager, L. P. *J. Org. Chem.* **1996**, *61*, 3923−5.
(5) Zaks, A.; Dodds, D. R. *J. Am. Chem. Soc.* **1995**, *117*, 10419−24.
(6) Miller, V. P.; Tschirret-Guth, R. A.; Ortiz de Montillano, P. R. *Arch. Biochem. Biophys.* **1995**, *319*, 333−40.
(7) (a) Libby, R. D.; Beachy, T. M.; Phipps, A. K. *J. Biol. Chem.* **1996**, *271*, 21820−7. (b) Fu, H.; Kondo, H.; Ichikawa, Y.; Look, G. C.; Wong, C.-H. *J. Org. Chem.* **1992**, *57*, 7265−70. (c) Libby, R. D.; Thomas, J. A.; Hager, L. P. *J. Biol. Chem.* **1982**, *257*, 5030−7. (d) Neidelman, S. L.; Geigert, J. *Biochem. Soc. Symp.* **1981**, *48*, 39−52. (e) Morrison, S. L.; Schonbaum, G. R. *Annu. Rev. Biochem.* **1976**, *45*, 861−89. (f) Hager, L. P.; Morris, D. R.; Brown, F. S.; Eberwein, H. *J. Biol. Chem.* **1966**, *241*, 1769−77.
(8) (a) Allenmark, S. G.; Andersson, M. A. *Tetrahedron: Asymmetry* **1996**, *7*, 1089−94. (b) Van Deurzen, M. P. J.; Groen, B. W.; Van Rantwijk, F.; Sheldon, R. A. *Biocatalysis* **1994**, *10*, 247−55. (c) Colonna, S.; Gaggero, N.; Casella, L.; Carrea, G.; Pasta, P. *Tetrahedron: Asymmetry* **1992**, *3*, 95−106. (d) Colonna, S.; Gaggero, N.; Manfred, A.; Casella, L.; Gullotti, M.; Carrea, G.; Pasta, P. *Biochemistry* **1990**, *29*, 10465−8. (e) Kobayashi, S.; Nakano, M.; Goto, T.; Kimura, T.; Schapp, A. P. *Biochem. Biophys. Res. Commun.* **1986**, *135*, 166−71.
(9) (a) Okazaki, O.; Guengerich, F. P. *J. Biol. Chem.* **1993**, *268*, 1546−52. (b) Ketters, G. L.; Hollenberg, P. F. *Arch. Biochem. Biophys.* **1984**, *233*, 315−321.
(10) Casella, L.; Poli, S.; Gullotti, M.; Selvaggini, C.; Beringhelli, T. *Biochemistry* **1994**, *33*, 6377−86.
(11) Geigert, J.; Dalietos, D. J.; Neidelman, S. L.; Lee, T. D.; Wadsworth, J. *Biochem. Biophys. Res. Commun.* **1983**, *114*, 1104−8.
(12) (a) Doerge, D. R.; Corbett, M. D. *Chem. Res. Toxicol.* **1991**, *4*, 556. (b) Corbett, M. D.; Chipko, B. R.; Batchelor, A. O. *Biochem. J.* **1980**, *187*, 893−903.
(13) Sundaramurthy, M.; Terner, J.; Poulos, T. L. *Structure* **1995**, *3*, 1367−77.
(14) (a) Fox, T.; Kollman, P. A. *J. Phys. Chem.* **1996**, *100*, 2950−6. (b) Ortiz de Montellano, P. R.; Choe, Y. S.; DePillis, G.; Catalano, C. E. *J. Biol. Chem.* **1987**, *262*, 11641−6. (c) For a general treatment of metalloporphyrin oxidation mechanisms, see: *Metalloporphyrins in Catalytic Oxidations*; Sheldon, R. A., Ed.; M. Dekker: New York, 1994.
(15) Colonna, S.; Gaggero, N.; Casella, L.; Carrea, G.; Pasta, P. *Tetrahedron: Asymmetry* **1993**, *4*, 1325−30.
(16) Sun, W.; Kadina, T. A.; Pickard, M. A.; Dunford, H. B. *Biochem. Cell Biol.* **1994**, *72*, 321−31.
(17) (a) Van Deurzen, M. P. J.; Van Rantwijk, F.; Sheldon, R. A. *J. Mol. Catal. B: Enzymatic* **1996**, *2*, 33−42. (b) Shevelkova, A. N.; Ryabov, A. D. *Biochem. Mol. Biol. Int.* **1996**, *39*, 665−70.

S0002-7863(96)02998-8 CCC: $14.00

Table 1. Chloroperoxidase-Mediated Epoxidation of ω-Bromo-2-methylalkenes: Conversions, Kinetic Parameters, and Enantioselectivities as a Function of Chain Length[a]



entry	substrate	ee[b] (%)	conv[b] (%)	yield[b] (%)	V_{max}[c] (μmol/min)	K_m[c] (mM)
1		62	100	61	—	—
2		88	100	93	3.5	3.7
3		95	65	89	0.62	0.86
4		87	30	33	0.21	0.42
5		50	20	42	—	—

[a] See text and Supporting Information for experimental details. [b] Determined by chiral GC analysis. [c] Data derived from Figure 1.

thermodynamically dangerous to its health. When oxidation of substrate is slow, the life span of the catalyst is short.

The crystal structure[13] of CPO reveals a catalytic heme functionality immersed in a proteinaceous pocket. Entry into this active site is likely to be increasingly inhibited toward substrates with greater steric requirements. Thus, it is likely that the slower rate of diffusion into the active site pocket for longer chain substrates is what allows for compound I self-destruction and, hence, lower conversions.

Poor solubilities of these substrates in water entail that preparatory scale reactions be conducted as two-phase systems. Kinetic experiments must be conducted at concentrations at and below maximum solubility, which did present difficulties, but approximate kinetic parameters could be obtained for several of the alkenes. In each case, a kinetic burst was observed which quickly leveled off to a steady-state reaction. Figure 1 illustrates Lineweaver−Burk plots using epoxide formation data collected from steady-state analyses. These plots provide the kinetic parameters V_{max} and K_m which corroborate faster reaction rates for shorter chain substrates while binding is somewhat enhanced for the longer chain alkenes (Table 1).

Like conversion data, enantiomeric excess data in Table 1 show an interesting trend. Methallyl bromide (entry 1) was epoxidized resulting in moderate enantioselectivity (62% ee) while the ee of the oxide of 4-bromo-2-methyl-1-butene was good (88%, entry 2) and that of 5-bromo-2-methyl-1-pentene was excellent (95%, entry 3). CPO proved to be progressively less stereoselective toward alkenes possessing additional carbons. 6-Bromo-2-methyl-1-hexene (entry 4) and 7-bromo-2-methyl-1-heptene (entry 5) provided epoxides with 87 and 50% ee, respectively.

The modest ee associated with the smallest substrate is reasonable considering that the plane of symmetry dividing each substituent on the double bond is disturbed only by a bromine atom which possesses a Van der Walls radius of 1.84 Å,[18]

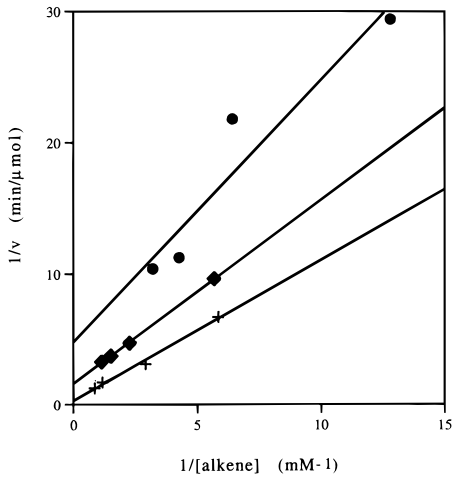


Figure 1. Lineweaver−Burk plots for several ω-bromo-2-methyl-1-alkenes; v is the velocity (μmol/min) of epoxide formation under steady-state conditions. Reaction mixtures contained 10 mM sodium citrate buffer, 0.18 μM CPO, 40 mM TBHP at 22 °C, and either 4-bromo-2-methyl-1-butene (+), 5-bromo-2-methyl-1-pentene (◆), or 6-bromo-2-methyl-1-hexene (●).

slightly less than that of a methyl group. As the chain lengthens, the nonsymmetry becomes more pronounced and enantioselectivity increases—but only to a point. Again, consideration of active site structural features can be informative. 2-Methyl-alkenes with especially long chains are liable to fold into higher energy conformations as required by the steric constraints of the active site pocket. Meanwhile, the energy difference between *re* and *si* orientation for oxygen transfer diminishes for these substrates resulting in lower ee values for epoxide products.

A question that remains is whether admission to the active site or the activation energy for epoxidation represents the higher energy barrier. Further research is expected to proceed involving reaction kinetic experiments, molecular modeling, and random and site-directed mutagenisis.

Acknowledgment. Chloroperoxidase was isolated by Annette Dexter from culture filtrates of *C. fumago* provided by Chirazyme Laboratories, 2004 S. Wright St., Urbana, IL 61801. This research was supported by a grant (GM-07768) from the National Institute of General Medical Sciences.

Supporting Information Available: Experimental procedures for kinetic experiments and the preparations of bromoalkenes, including spectroscopic data and chiral GLC data of the epoxides (10 pages). See any current masthead page for ordering and Internet access instructions.

JA962998X

(18) Bondi, A. *J. Phys. Chem.* **1964**, *68*, 441−51.